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Impact of level and timing of pruning on flower yield and secondary metabolites profile of *Rosa damascena* under western Himalayan region

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ABSTRACT

Pruning has been implicated as a promoter of bud outgrowth or shoot branching and flower yield in *Rosa damascena* Mill., but the extent and time of pruning are tightly regulated by the climatic conditions of the growing region. In western Himalaya region in India, the effect of extent and time of pruning on flower yield and secondary metabolites of *R. damascena* is still unsolved. Thus, a field experiment comprising three levels of pruning and four different time of pruning was conducted to confirm that the higher yield and quality can be achieved through the standardization of pruning practices. Principal component analysis showed that pruning at 90 cm height from ground level (FGL) on 15th December is preferable for higher flower yield and essential oil. The yield data suggest that moderate pruning (90 cm FGL) leads to an increased rate of flower production (11.33 and 13.22 g new shoot^{-1}). Pruning on15th December produced 10.6–42.77% higher flower yield compared with pruning on 31st October. The quality of oil is considerably affected by level and time of pruning. The results have suggested that the higher yield and quality of *R. damascena* can be achieved through the standardization of pruning practices.

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1. Introduction

Rosa damascena Mill., the essential oil bearing rose, is an important industrial crop cultivated for its high-value essential oil in different parts of the world. *R. damascena* Mill., usually known as Damask rose, is extensively cultivated for essential oil and medicinal aspects in Bulgaria, Turkey, India and Iran (Tabaei-Aghdaei et al., 2006). Bulgaria and Turkey are the main producers of rose essential oil, and whole rose industries in Bulgaria and Turkey are based on a single genotype which has been vegetatively propagated for centuries (Rusanov et al., 2005, 2009). Rose oil is the most expensive essential oil in the World market that is why it is called as 'liquid gold'. Essential oil of rose is extensively used in perfumery and cosmetic industry as a base constituent for its strong fragrance. In addition to its uses in aromatic industries, some important characteristics of *R. damascena* oil such as antibacterial, anti-infective and anti-inflammatory (Basim and Basim, 2003) and antioxidant (Ozkan et al., 2004) properties have been reported. The by-product of oil-bearing rose, a large scale waste, is also a source of natural antioxidant (Baydar and Baydar, 2013).

The quality of industrial products (essential oil, concrete, absolute and rose water) of *R. damascena* varies according to genetic make-up, environmental conditions, agronomic management and distillation methods (Baydar and Baydar, 2005; Shawl and Adams, 2009; Pal, 2013). In rose oil, the percentage of major components is one of the important parameters to determine the quality (Boelens and Boelens, 1997). Flower, the harvestable part of *R. damascena*, is distilled for essential oil. In the western Himalayan region in India, the blooming of *R. damascena* happens once a year in the 1st week of April, and the duration of flowering period is about 35–45 days.

The yield of flower is greatly influenced by the agronomic practices. Among the agronomic practices of *R. damascena*, pruning is the most important practice for determining the flower yield. The pruning of rose, a horticultural art for manipulating plant architecture, depends on types of rose to be pruned and purpose of pruning (Pal and Singh, 2013). Generally, the main objectives of pruning in rose are to manipulate branching and flower production in different seasons. This agronomic practice changes the plant source–sink ratio, which can modify canopy gas-exchange capacity (Medhurst et al., 2006). Calatayud et al. (2008) also reported that pruned plant has higher capacity to promote the photosynthetic light reaction, a

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large number of metabolic sinks and a higher turgor pressure compared with non-pruned plants. Nevertheless, the older leaf might have different responses to irradiation compared to younger leaf, as it occurred in coffee (Wareing et al., 1968). Annual pruning is necessary for promoting branches and for providing proper shape of bushes to make easy for agronomic practices. The level and time of pruning are the important yield determinants of *R. damascena*. However, extent and time of pruning depend on agronomic management, cultivars and the climatic conditions of the growing region.

Astadzhov et al. (1986) reported that, in Bulgaria, the light to moderate pruning increased the flower yield and flower oil content, whereas heavy pruning reduced both. It has been also reported that light and medium pruning are better than the heavy pruning in terms of petals weight and oil content, respectively (Hassanein, 2010). Hence, inappropriate level with wrong time of pruning could reduce the flower yield and quality of essential oil of *R. damascena*. The proper level and time of pruning may be contemplated as a means of addressing this issue in these situations. Therefore, it becomes imperative to standardize the level and time of pruning, so that the farmers can harvest maximum flowers, which contain high quality essential oil. Several studies have been conducted on harvest management, post-harvest technology, distillation methods, chemical composition of essential oil, physiological and agronomic aspects of *R. damascena* (Baydar and Baydar, 2005; Shawl and Adams, 2009; Kazaz et al., 2010; Dobreva et al., 2011; Rusanov et al., 2011, 2012).

The effect of the severity and time of pruning on growth and flower yield has been studied in greenhouse conditions. Nevertheless, no studies have been initiated to standardize the level and time of pruning for attaining the higher flower yield and quality of essential oil of *R. damascena* under open field condition in western Himalaya region. Our objective in the present study was to test the hypothesis that proper level and time of pruning could enhance the productivity of *R. damascena* in terms of flower yield and quality of essential oil. For this purpose, the plants were pruned at 60, 90 and 120 cm height from ground level (FGL), and the pruning operations were done on four different days (31st October, 15th November, 30th November and 15th December) during 2010 and 2011. Then, we investigated the effects of level of pruning and time of pruning on growth, physiological attributes, flower yield, oil content and composition of essential oil of *R. damascena*.

2. Materials and methods

2.1. Experimental location, climate and soil characteristics

The present investigation was carried out at the experimental farm of CSIR-Institute of Himalayan Bioresource Technology, Palampur, India, during the growing seasons of 2010–2011 and 2011–2012, to standardize the level and time of pruning for attaining higher yield and quality of essential oil of *R. damascena* under western Himalayan region. The experimental site falls within latitude of 32°06′05″ N and longitude of 76°34′10″ E, and altitude of 1395 m above mean sea-level. The mean annual rainfall of Palampur is 2500 mm, and more than 65% of the total rainfall receives during the south-west monsoon season (July–September) with annual mean temperature of 18 °C. Data on weather parameters *viz*., maximum and minimum temperature, relative humidity, sunshine hours and rainfall during the crop seasons were presented in Fig. 1. The soil of experimental field was silty clay in texture. Soil pH was 6.14 (1:2.5). Available nitrogen (N), phosphorus (P) and potassium (K) in the soil were 335.5, 28.3 and 349.3 kg ha^{-1}, respectively.

2.2. Plant material, crop management and application of treatments

Three years old plantation of *R. damascena* (*cv*. Jwala) field was selected for this investigation. The planting geometry was 0.75 m within rows and 1.5 m between rows. The experimental field was fertilized with nitrogen (N), phosphorus (P) and potassium (K) at the rate of 200, 43.7 and 83 kg ha^{-1}, respectively during the investigating years. Half dose of N and full dose of P and K were applied at the time of pruning, while the remaining half quantity of N was applied at 20th day after pruning. Other recommended agronomic practices for *R. damascena* were adopted during the course of study. The experiment was designed as two factor factorial arrangement in randomized block design (RBD) with three replications. Twelve treatment combinations comprising three levels of pruning (60, 90 and 120 cm height from ground level) and four different time of pruning (31st October, 15th November, 30th November and 15th December) were tested.

2.3. Extraction of essential oil

The essential oil of the fresh harvested flower was extracted by hydro-distillation using a Clevenger-type apparatus. The flower and water ratio was 1:3 (w/v), and the distillation time was 4 h. The oil content (w/w) in flower was determined as percentage on a fresh weight basis. The extracted oil samples were dehydrated by anhydrous sodium sulphate (Merck) and stored in a dark place at 4 °C until analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS).

2.4. GC analysis and quantification

Gas chromatographic analysis was performed by a Shimadzu GC-2010 gas chromatograph (Shimadzu, Tokyo, Japan) equipped with flame ionization detector (FID) and a DB-5 capillary column (30 m × 0.25 mm, fused silica, and film thickness 0.25 μm). The operating conditions were as follows: the oven temperature programmed from 70 °C (4 min) to 220 °C with stepwise increase at the rate of 4 °C min^{-1} and held for 5 min, injector and detector temperature set at 240 °C and 250 °C, respectively; nitrogen as the carrier gas with a velocity of 1.05 mL min^{-1}. The compounds were quantified by peak area normalization, and the response factor for each component was considered equal to 1.

2.5. GC–MS analysis

All the GC–MS analysis of flower extracts were carried out by a Shimadzu QP2010 GC–MS system (Shimadzu, Tokyo, Japan) coupled with AOC-5000 auto injector and DB-5 (SGE International, Ringwood, Australia) fused silica capillary column (30 m × 0.25 mm i.d., and film thickness 0.25 μm). The temperature was programmed from 70 °C (4 min) to 220 °C (5 min) with stepwise increasing at the rate of 4 °C min^{-1} and held for 5 min; injector and interface temperatures were 240 °C and 250 °C, respectively; ionization voltage was 70 eV with acquisition mass range, 800–50 amu. Helium was used as a carrier gas with a flow rate of 1.1 mL min^{-1}.

2.6. Compound identification

The retention indices (RI) for all volatile components were calculated using homologous series of *n-alkanes* (C_8–C_{24}). The components of oil were identified based on a comparison of RI and mass spectra with NIST-MS (National Institute of Standards and Technology-mass spectral) library (Stein, 2005).

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